ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS

AB The present inventors have characterized the surface antigens of the bacterial pathogen P. salmonis and identified and characterized an immunoreactive antigen, namely the **17 kDa** outer surface lipoprotein OspA of P. salmonis, as well as the nucleic acid segment that encodes the OspA immunoreactive antigen. In particular, this invention relates to the use of the **17 kDa** outer surface lipoprotein (OspA) of **Piscirickettsia salmonis**, or its homologues, as the basis of, or part thereof, a recombinant vaccine for salmonid rickettsial septicemia and other rickettsial diseases. This invention also relates to the augmentation of protective immunity by the inclusion of promiscuous T lymphocyte epitopes (TCE's) in fusion protein constructs in salmonids. This invention also relates to the use of bacterial protein inclusion bodies as a source of the protective immunogen.

AN 2001:652901 CAPLUS

DN 135:256116

TI Sequences of **Piscirickettsia salmonis** lipoprotein (OspA), vaccine for inducing immunity against P. salmonis and other rickettsial diseases, and incorporation of T lymphocyte epitopes (TCE's) to enhance immunological response in fish

IN Burian, Jan; Kuzyk, Michael A.; Thornton, Julian C.; Kay, William W.

PA University of Victoria Innovations Dept., Can.

SO Brit. UK Pat. Appl., 59 pp.
CODEN: BAXXDU

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	GB 2356632	A1	20010530	GB 2000-22825	20000918
	NO 2000004637	A	20010319	NO 2000-4637	20000915
PRAI	CA 1999-2281913	A	19990917		
	US 1999-143437P	P	19990917		

L9 ANSWER 2 OF 3 USPATFULL

AB A system for the generation of live, nonpathogenic infectious pancreatic necrosis virus (IPNV), a segmented double-stranded (ds)RNA virus of the Birnavirdae family, using synthetic transcripts derived from cloned DNA has been developed. Independent full-length cDNA clones were constructed which contained the coding and non-coding regions of RNA segments A and B of IPNV, respectively. Segment A was modified to prevent the expression of NS protein. Synthetic RNAs of both segments were produced by in vitro transcription of linearized plasmids with T7 RNA polymerase. Transfection of CHSE cells with combined plus-sense transcripts of both segments generated infectious virus. The development of a system for producing NS protein deficient IPNV will greatly facilitate studies of viral pathogenesis, and the development of live attenuated vaccines for IPNV.

AN 2001:130869 USPATFULL

TI Method for generating nonpathogenic infectious pancreatic necrosis virus (IPNV) from synthetic RNA transcripts

IN Vakharia, Vikram N., Bowie, MD, United States
Yao, Kun, College Park, MD, United States

PA University of Maryland-Biotechnology Institute, College Park, MD, United States (U.S. corporation)

PI US 6274147 B1 20010814

AI US 1999-282147 19990331 (9)

PRAI US 1998-80178P 19980331 (60)

DT Utility

FS GRANTED

EXNAM Primary Examiner: Mosher, Mary E.

LREP Arent Fox Plotkin Kintner Kahn PLLC.

CLMN Number of Claims: 24

ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 1615
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AB No effective recombinant vaccines are currently available for any rickettsial diseases. In this regard the first non-ribosomal DNA sequences from the obligate intracellular pathogen **Piscirickettsia salmonis** are presented. Genomic DNA isolated from Percoll density gradient purified P. salmonis, was used to construct an expression library in lambda ZAP II. In the absence of preexisting DNA sequence, rabbit polyclonal antiserum raised against P. salmonis, with a bias toward P. salmonis surface antigens, was used to identify immunoreactive clones. Catabolite repression of the lac promoter was required to obtain a stable clone of a 4,983 bp insert in Escherichia coli due to insert toxicity exerted by the accompanying radA open reading frame (ORF). DNA sequence analysis of the insert revealed 1 partial and 4 intact predicted ORF's. A 486 bp ORF, ospA, encoded a **17 kDa** antigenic outer surface protein (OspA) with 62% amino acid sequence homology to the genus common **17 kDa** outer membrane lipoprotein of Rickettsia prowazekii, previously thought confined to members of the genus Rickettsia. Palmitate incorporation demonstrated that OspA is posttranslationally lipidated in E. coli, albeit poorly expressed as a lipoprotein even after replacement of the signal sequence with the signal sequence from lpp (Braun lipoprotein) or the rickettsial **17 kDa** homologue. To enhance expression, ospA was optimized for codon usage in E. coli by PCR synthesis. Expression of ospA was ultimately improved (apprx13% of total protein) with a truncated variant lacking a signal sequence. High level expression (apprx42% tot. prot.) was attained as an N-terminal fusion protein with the fusion product recovered as inclusion bodies in E. coli BL21. Expression of OspA in P. salmonis was confirmed by immunoblot analysis using polyclonal antibodies generated against a synthetic peptide of OspA (110-129) and a strong antibody response against OspA was detected in convalescent sera from coho salmon (Oncorhynchus kisutch).
AN 2002:147899 BIOSIS
DN PREV200200147899
TI OspA, a lipoprotein antigen of the obligate intracellular bacterial pathogen **Piscirickettsia salmonis**.
AU Kuzyk, Michael A.; Burian, Jan; Thornton, Julian C.; Kay, William W. (1)
CS (1) Department of Biochemistry and Microbiology, Canadian Bacterial Diseases Network, University of Victoria, Victoria, BC, V8W 3P6: wkay@uvic.ca Canada
SO Journal of Molecular Microbiology and Biotechnology, (January, 2001) Vol. 3, No. 1, pp. 83-93. http://www.jmmb.net. print.
ISSN: 1464-1801.
DT Article
LA English

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L11 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS

AB The present inventors have characterized the surface antigens of the bacterial pathogen P. salmonis and identified and characterized an immunoreactive antigen, namely the 17 kDa outer surface lipoprotein **OspA** of P. salmonis, as well as the nucleic acid segment that encodes the **OspA** immunoreactive antigen. In particular, this invention relates to the use of the 17 kDa outer surface lipoprotein (**OspA**) of **Piscirickettsia salmonis**, or its homologues, as the basis of, or part thereof, a recombinant vaccine for salmonid rickettsial septicemia and other rickettsial diseases. This invention also relates to the augmentation of protective immunity by the inclusion of promiscuous T lymphocyte epitopes (TCE's) in fusion protein constructs in salmonids. This invention also relates to the use of bacterial protein inclusion bodies as a source of the protective immunogen.

AN 2001:652901 CAPLUS

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TI Sequences of **Piscirickettsia salmonis** lipoprotein (**OspA**), vaccine for inducing immunity against P. salmonis and other rickettsial diseases, and incorporation of T lymphocyte epitopes (TCE's) to enhance immunological response in fish

IN Burian, Jan; Kuzyk, Michael A.; Thornton, Julian C.; Kay, William W.

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FAN.CNT 2

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	NO 2000004637	A	20010319	NO 2000-4637	20000915
PRAI	CA 1999-2281913	A	19990917		
	US 1999-143437P	P	19990917		

L11 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

AB **Piscirickettsia salmonis** is the aetiological agent of salmonid rickettsial septicaemia, an economically devastating rickettsial disease of farmed salmonids. Infected salmonids respond poorly to antibiotic treatment and no effective vaccine is available for the control of P. salmonis. Bacterin preparations of P. salmonis were found to elicit a dose-dependent response in coho salmon (Oncorhynchus kisutch), which varied from inadequate protection to exacerbation of the disease. However, an outer surface lipoprotein of P. salmonis, **OspA**, recombinantly produced in Escherichia coli elicited a high level of protection in vaccinated coho salmon with a relative percent survival as high as 59% for this single antigen. In an effort to further improve the efficacy of the **OspA** recombinant vaccine, T cell epitopes (TCE's) from tetanus toxin and measles virus fusion protein, that are universally immunogenic in mammalian immune systems, were incorporated tandemly into an **OspA** fusion protein. Addition of these TCE's dramatically enhanced the efficacy of the **OspA** vaccine, reflected by a three-fold increase in vaccine efficacy. These results represent a highly effective monovalent recombinant subunit vaccine for a rickettsia-like pathogen, P. salmonis, and for the first time demonstrate the immunostimulatory effect of mammalian TCE's in the salmonid immune model. These results may also be particularly pertinent to salmonid aquaculture in which the various subspecies are outbred and of heterologous haplotypes.

AN 2001:188012 BIOSIS

DN PREV200100188012

TI An efficacious recombinant subunit vaccine against the salmonid rickettsial pathogen **Piscirickettsia salmonis**.

AU Kuzyk, Michael A.; Burian, Jan; Machander, Dave; Dolhaine, Daphne; Cameron, Stephen; Thornton, Julian C.; Kay, William W. (1)

CS (1) Canadian Bacterial Diseases Network, Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, V8W 3P6: wkay@uvic.ca Canada
SO Vaccine, (21 March, 2001) Vol. 19, No. 17-19, pp. 2337-2344. print. ISSN: 0264-410X.
DT Article
LA English
SL English

L11 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AB No effective recombinant vaccines are currently available for any rickettsial diseases. In this regard the first non-ribosomal DNA sequences from the obligate intracellular pathogen **Piscirickettsia salmonis** are presented. Genomic DNA isolated from Percoll density gradient purified P. salmonis, was used to construct an expression library in lambda ZAP II. In the absence of preexisting DNA sequence, rabbit polyclonal antiserum raised against P. salmonis, with a bias toward P. salmonis surface antigens, was used to identify immunoreactive clones. Catabolite repression of the lac promoter was required to obtain a stable clone of a 4,983 bp insert in Escherichia coli due to insert toxicity exerted by the accompanying radA open reading frame (ORF). DNA sequence analysis of the insert revealed 1 partial and 4 intact predicted ORF's. A 486 bp ORF, **ospA**, encoded a 17 kDa antigenic outer surface protein (**OspA**) with 62% amino acid sequence homology to the genus common 17 kDa outer membrane lipoprotein of Rickettsia prowazekii, previously thought confined to members of the genus Rickettsia. Palmitate incorporation demonstrated that **OspA** is posttranslationally lipidated in E. coli, albeit poorly expressed as a lipoprotein even after replacement of the signal sequence with the signal sequence from lpp (Braun lipoprotein) or the rickettsial 17 kDa homologue. To enhance expression, **ospA** was optimized for codon usage in E. coli by PCR synthesis. Expression of **ospA** was ultimately improved (apprx13% of total protein) with a truncated variant lacking a signal sequence. High level expression (apprx42% tot. prot.) was attained as an N-terminal fusion protein with the fusion product recovered as inclusion bodies in E. coli BL21. Expression of **OspA** in P. salmonis was confirmed by immunoblot analysis using polyclonal antibodies generated against a synthetic peptide of **OspA** (110-129) and a strong antibody response against **OspA** was detected in convalescent sera from coho salmon (Oncorhynchus kisutch).
AN 2002:147899 BIOSIS
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AU Kuzyk, Michael A.; Burian, Jan; Thornton, Julian C.; Kay, William W. (1)
CS (1) Department of Biochemistry and Microbiology, Canadian Bacterial Diseases Network, University of Victoria, Victoria, BC, V8W 3P6: wkay@uvic.ca Canada
SO Journal of Molecular Microbiology and Biotechnology, (January, 2001) Vol. 3, No. 1, pp. 83-93. http://www.jmmb.net. print. ISSN: 1464-1801.
DT Article
LA English

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   (FILE 'HOME' ENTERED AT 15:51:40 ON 11 MAR 2003)

     FILE 'BIOSIS, CABA, CAPLUS, EMBASE, LIFESCI, MEDLINE, SCISEARCH,
     USPATFULL, JAPIO' ENTERED AT 15:51:51 ON 11 MAR 2003
L1           253 S (OUTER SURFACE LIPOPROTEIN)
L2          9447 S (17 KDA OR 17 KILODALTON)
L3             1 S L1 AND L2
L4          4018 S OSP A  OR OSPA
L5            34 S L4 AND L2
L6            17 DUP REM L5 (17 DUPLICATES REMOVED)
L7           219 S  PISCIRICKETTSIA SALMONIS
L8             9 S L7 AND L2
L9             3 DUP REM L8 (6 DUPLICATES REMOVED)
L10           15 S L4 AND L7
L11            3 DUP REM L10 (12 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 16:00:38 ON 11 MAR 2003

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